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On December 7, 2005

TOWNSEND and TOWNSEND and CREW LLP

By: _____
Lois M. Simón

AMENDMENT UNDER 37 CFR 1.116
EXPEDITED PROCEDURE –
EXAMINING GROUP 1651

PATENT
Docket No.: 002558-064410US
Client Ref. No.: BRP0098

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Quan Nguyen

Application No.: 10/092,926

Filed: March 6, 2002

For: ASSAY SYSTEM FOR SIMULTANEOUS DETECTION AND MEASUREMENT OF MULTIPLE MODIFIED CELLULAR PROTEINS

Examiner: Counts

Art Unit: 1641

DECLARATION UNDER 37 C.F.R. 1.132

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QUAN NGUYEN declares and states:

1. I attended University of California, Berkeley from 1982-1987, and have to complete a history class to obtain by Bachelor of Science in Molecular biology. I am one of the inventors in this patent application.

2. I have approximately 10 years of experience in the field of immunoassays, including having worked on systems, kits, and procedures for conducting simultaneous assays for detection and/or determination of multiple target analytes (so-called "multiplex" assays).

3. The invention disclosed and claimed in this patent application is a method for simultaneous detection and/or determination of two or more modified proteins in a sample, and includes the step of contacting the sample with a group of two or more first antibodies under what we consider mild protein denaturation conditions. As stated in claim 1, the mild denaturation conditions include a temperature of between about 4 and about 37 °C, a contacting time of about 2 to about 72 hours, and the use of about 1-10 mM concentration of a sulfate or sulfonate detergent.

4. The modified proteins can include for example, phosphorylated, glycosylated, acetylated, methylated, ubiquinated, and prenylated proteins. A preferred detergent is SDS (sodium dodecyl sulfate).

5. In preparing the patent application we were aware of U.S. patent 4,658,022, which is discussed in the patent application on page 5. This patent describes a process for analyzing for a single modified protein, where the modification may be one of the types mentioned above, by contacting the protein with an antibody and a detergent such as SDS under protein denaturation conditions that are much harsher than those we use, namely at higher temperatures (above 50 °C) and much shorter times (one minute or less). This technique is described in that patent at column 8 lines 42 through 67.

6. While this procedure may be satisfactory for analysis of a single modified protein, it is not appropriate for use in simultaneous analysis of a mixture of modified proteins because of the potential for partial or complete denaturation of the capture antibodies that are added in the same cell lysis buffer. The completely denatured antibodies would not be functional and would not bind to any targets. The partially denatured antibodies would bind to many non-specific targets, instead of binding to a specific target. In the multiplex antibody-based assays, there are more antibodies used; hence, more chances for antibodies to be completely or partially denatured. In addition, although used in that patent, SDS itself is generally considered to be too strong a detergent for use in the same mixture with antibodies. SDS is a potent detergent commonly used in Western blotting procedure to unfold and linearize proteins from a three dimensional, folded native structure into a linear chain of polypeptides. In the Western blotting procedure, the proteins must be unfolded and linearized in order to be separated by their molecular weight in the denaturing polyacrylamide gel electrophoresis. Once the linearized protein is transferred to a nylon membrane, SDS is completely washed out, removed before probing the protein with the specific antibody and never present during the incubation with the antibody. Therefore, it is a surprise that we were able to get the multiplex protein assay to work in the presence of SDS and antibodies in the same reaction.

7. It thus was surprising to us, and would have been surprising to other researchers, that SDS could be used in a multiplex assay for modified proteins under appropriate conditions, namely those that are claimed in this patent application - a relatively low concentration, a relatively low temperature, and a relatively long time - that is, relatively mild conditions.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the patent to which this verified states is directed.

Quan Nguyen

Dec/05/2005

Date

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